CONCISE COMMUNICATION

Identification of *Aerococcus urinae* in urine samples

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To evaluate procedures for the identification of *Aerococcus urinae*, we examined 24 α-hemolytic non-enterococcal bacterial isolates from 4373 urine samples. Published procedures were compared with 16s rRNA sequencing and biochemical profiling (BBL-Crystal-GP). 16s rRNA sequencing and BBL-Crystal-GP identified the same 13 isolates as *A. urinae*. Published tests failed to distinguish the 13 *A. urinae* isolates from eight non-*A. urinae* isolates; several tests exhibited no discrimination. Ciprofloxacin and trimethoprim susceptibility and growth at 45 °C improved discrimination. For urinary isolates, standard procedures for identification of *A. urinae* are redundant and insufficiently discriminatory, and may need revision. BBL-Crystal-GP is an accurate alternative.

Keywords *Aerococcus urinae*, α-hemolytic streptococci, urinary tract infection

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In 1989, Christensen et al. [1] described a urinary pathogen which they named '*Aerococcus*-like organism (ALO)'. The bacterium was later named *Aerococcus urinae* [2]. This organism was subsequently reported in urinary infections in The Netherlands and the USA [3,4], and it was noted that serious complications in the form of septicemia and endocarditis may arise from such infections [5,6]. Children and the elderly are most prone to infection [7], and chronic diseases such as diabetes mellitus, cancer and prostatic hyperplasia are predisposing factors. Korner and Christensen define *A. urinae* as an α-hemolytic Gram-positive coccus occurring in pairs or tetrads, similar to staphylococci; it is catalase negative, negative for pyrrolidinyl peptidase (PYR), positive for leucine aminopeptidase (LAP), susceptible to penicillins, resistant to sulfonamides, and moderately or fully resistant to gentamicin [7]. *A. urinae* is further described as ciprofloxacin susceptible, showing full or moderate resistance to trimethoprim, and showing growth at 45 °C. This study was initiated to investigate the presence of *A. urinae* in clinical urinary specimens from Norway, and to evaluate the published methods for its identification [7,8].

During the period 1 October 2000 to 31 December 2000, our laboratory (covering 165 000 inhabitants out of a total population in Norway of 4.5 million) received 4373 urine samples, of which 2443 (55.9%) showed significant bacteriuria ($\geq 10^4$ CFU/mL in pure culture, or dominant growth with no more than one additional bacterium present). Thirty-three isolates were initially identified as possibly *A. urinae*, being α-hemolytic streptococci with staphylococcoid microscopic morphology but lacking the characteristic shiny colony morphology and malty odor of enterococci. These isolates were kept frozen at −70 °C until further analysis. On re-examination, nine isolates were excluded due to non-viability, impurity, lack of α-hemolysis, or failure to grow on antibiotic susceptibility test media. The remaining 24 isolates were from 19 patients (16 female and three male). All isolates were investigated for catalase, bacterial morphology on Gram-stained preparations, PYR (Murex Biotech, Dartford, UK), growth on blood agar at 45 °C, LAP (A/S Roscoe, Taastrup, Denmark), susceptibility to gentamicin, ampicillin, vancomycin and sulfonamide by the disk diffusion test (Mast Ltd, Merseyside, UK), and susceptibility to gentamicin, trimethoprim and ciprofloxacin by the Etest (AB Biodisk, Solna, Sweden). Except where otherwise specified, bacteria were cultured at 37 °C for 16–18 h in a 10% CO_2 atmosphere.

Biochemical profiles were determined with the BBL Crystal-GP biochemical identification kit (Becton-Dickinson, Sparks, MD, USA), a proprietary panel of 30 biochemical and sugar fermentation tests. Sequences of the 16s rRNA gene (260–350 bp) were kindly determined by Dr Dominique Caugant of the National Institute of Public Health, Oslo, using a modification of the method of Rogall et al. [9].

The results are summarized in Table 1.

Sequencing of the 16s rRNA gene identified 13 isolates as *A. urinae*. The same isolates were identified as *A. urinae* using the BBL-Crystal-GP bacterial identification system. Microscopic examination of Gram-stained preparations showed all isolates to be Gram-positive cocci in pairs or tetrads. All isolates were catalase negative, negative for PYR, susceptible to ampicillin and vancomycin, and resistant to sulfonamides. All of these isolates were resistant to gentamicin by the Etest, but six were scored as gentamicin susceptible by the disk diffusion assay. The isolates showed full or intermediate resistance to trimethoprim and ciprofloxacin, and grew at 45 °C. The Roscoe LAP test proved difficult to read, and was inconclusive; only one *A. urinae* isolate gave a clear positive reaction. The BBL-Crystal-GP bacterial identification system includes a comparable test, hydrolysis of proline- and leucine-p-nitroanilide (P/LAP), which will also detect the presence of proline aminopeptidase. All *A. urinae* isolates were positive according to this test.

16s rRNA and Crystal-GP identified the remaining 11 isolates as non-*A. urinae* spp., mostly members of the genus *Streptococcus*. For these non-*A. urinae* isolates, there was poor correlation between the results of 16s rRNA sequencing and Crystal-GP. Microscopic examination of Gram-stained preparations showed all isolates to be Gram-positive cocci in pairs or tetrads. All isolates were catalase negative, negative for PYR, susceptible to ampicillin and vancomycin, and resistant to sulfonamides. One isolate was susceptible to gentamicin, two were P/LAP negative, three were

Table 1 Age and sex of patients, species identification by 16s rRNA sequencing (16s), Crystal-BBL, and determination of selected characteristics

Patient	Age	Sex	GM	P/LAP	CI	TR	Growth at 45 °C	16s	Crystal
1	93	F	R	+	S	R	+	*A. urinae*	*A. urinae*
2	91	F	R	+	S	R	+ + +	*A. urinae*	*A. urinae*
2	91	F	R	+	S	R	+ + +	*A. urinae*	*A. urinae*
3	89	F	R	+	S	R	+ + +	*A. urinae*	*A. urinae*
4	86	F	R	0[a]	I	R	+ + +	*A. urinae*	*A. urinae*
4	86	F	R	+	I	R	+ + +	*A. urinae*	*A. urinae*
4	86	F	R	+	I	R	+ + +	*A. urinae*	*A. urinae*
5	86	F	R	0[a]	I	R	+ + +	*A. urinae*	*A. urinae*
6	83	F	R	+	S	I	+ + +	*A. urinae*	*A. urinae*
7	79	F	R	+	I	R	+ + +	*A. urinae*	*A. urinae*
7	79	F	R	+	S	R	+ + +	*A. urinae* (99)	*A. urinae*
8	79	F	R	0[a]	I	R	+ + +	*A. urinae*	*A. urinae*
9	73	F	R	+	S	R	+ + +	*A. urinae*	*A. urinae*
10	38	F	R	+	I	R	+ + +	**UOB**	***S. salivarius***
11	22	F	R	+	I	R	−	***S. sanguis***	***Pediococcus pentosaceus***
12	79	F	R	+	I	**S**	+ + +	***S. anguinosus* (99)**	***S. salivarius***
13	35	M	R	+	I	**S**	−	**UOB**	***S. mitis***
14	10	F	R	+	I	**S**	+ + +	***Streptococcus* sp.**	***S. parasanguinis***
15	75	M	R	+	**R**	**I**	+ + +	***S. oralis***	***S. parasanguinis***
16	64	F	R	+	**R**	**S**	+ + +	***S. oralis* (99)**	***S. parasanguinis***
12	89	F	R	+	**R**	**R**	−	***Actinobacillum schallii* (99)**	***Micrococcus luteus***
17	83	M	ND	−	**ND**	**ND**	+ + +	***S. fecalis***	***Enterococcus solitarius***
18	82	F	R	−	**I**	**R**	+ + +	**UOB**	***S. pneumoniae***
19	80	F	**S**	+	**I**	**R**	+ + +	**UOB**	***S. intermedius***

TR, trimethoprim; GM, gentamicin; CI, ciprofloxacin; P/LAP, hydrolysis of proline- and leucine-p-nitroanilide (proline/leucine aminopeptidase); R, resistant; S, susceptible; I, intermediate; UOB, unidentified oral bacterium; ND, not determined. Numbers in parentheses after 16s rRNA sequence identification indicate level of homology. Where not specified, this was 100%. Bold type indicates cumulative discrimination of non-*A. urinae* isolates on the basis of successive tests from left to right.

[a]Results of Crystal-GP were processed digitally; P/LAP results could not be recovered.

resistant to ciprofloxacin, three were susceptible to trimethoprim, and three failed to grow at 45 °C. One isolate was gentamicin resistant, P/LAP positive, ciprofloxacin intermediate, trimethoprim resistant, and grew at 45 °C, making it indistinguishable from *A. urinae*; the remaining three isolates could be distinguished from *A. urinae* on the basis of this combination of tests. Only three isolates could be distinguished from *A. urinae* on the basis of gentamicin susceptibility and P/LAP.

In all, 21 isolates were α-hemolytic Gram-positive cocci in pairs or tetrads, similar to staphylococci, catalase negative, negative for PYR, positive for P/LAP, susceptible to ampicillin, resistant to sulfonamides, and moderately or fully resistant to gentamicin. Only 13 of these isolates were *A. urinae*.

The 13 *A. urinae* isolates were from nine patients, all of whom were women over 70 years of age. In one patient, three urine samples collected over a period of 10 months contained *A. urinae*, confirming this organism's role as a cause of persistent urinary tract infection. The 11 non-*A. urinae* isolates were from 11 patients, three male and eight female, with ages in the range 10–89 years (mean age 60 years). For one patient, one sample contained *A. urinae*, while a second sample contained a non-*A. urinae* α-hemolytic isolate.

Twenty-one of the 24 isolates in this study satisfied the criteria of Korner and Christensen, of which only 13 were confirmed as *A. urinae* by 16s rRNA sequencing. However, as we were unable to obtain interpretable results from the Roscoe LAP test, we were obliged to substitute the hydrolysis of P/LAP. This may have reduced the specificity by including proline aminopeptidase-positive isolates. The BBL-Crystal-GP biochemical identification kit correctly and specifically identified all *A. urinae* isolates when 16s rRNA sequence determination was used as the standard.

We found that microscopic morphology, catalase, vancomycin, ampicillin and sulfonamide susceptibility and PYR were redundant tests that failed to discriminate between *A. urinae* and other α-hemolytic streptococci. Gentamicin susceptibility excluded only one non-*A. urinae* isolate, and in addition could not be reliably determined by the disk diffusion assay, which would have erroneously excluded six of the 13 *A. urinae* isolates identified in this study. However, a combination of susceptibility to gentamicin, ciprofloxacin and trimethoprim, P/LAP and growth at 45 °C approached the performance of 16s rRNA sequencing and Crystal-GP. Our results suggest that defining *A. urinae* as α-hemolytic, gentamicin resistant, moderately or fully susceptible to ciprofloxacin, moderately or fully resistant to trimethoprim, P/LAP or LAP positive and growing at 45 °C would improve discrimination of this species among urinary isolates, although this needs to be confirmed with a larger collection of isolates.

Over the 3-month period of this study, we found *A. urinae* in 13 of 4373 (0.3%) urine samples, or 13 of 2443 (0.53%) samples showing significant bacteruria, which is comparable with findings of 0.2–0.3% in The Netherlands [3], but lower than the 0.8% found in Denmark [7]. All *A. urinae* infections in this study were found in elderly women.

A. urinae urinary tract infections may lead to serious complications if untreated. It is thus important that routine microbial diagnostic procedures should be able to identify this bacterium and distinguish it from other bacteria with which it may be confused. Our findings suggest that there may be a need to reassess current recommendations for discrimination of *A. urinae* in urinary tract isolates. We suggest that greater importance should be assigned to growth at 45 °C, trimethoprim susceptibility, and LAP, while some of the redundant and non-discriminatory tests (microscopic morphology, catalase, PYR, vancomycin, ampicillin) may be safely excluded. We further advise against the disk diffusion test for gentamicin susceptibility as a criterion for identification, as the results are frequently incorrect.

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